Overview of the Ohio Wastewater Monitoring Network

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Ohio Wastewater Monitoring Network (OWMN)

**Goal**
- Monitor trend of SARS-CoV-2 RNA at specific locations (vs compare sites)
- Serve as early indicator of COVID-19 community spread
- Prioritize resources

**Statewide network**
- Started July 2020
- leveraged expertise and resources
  - Ohio Universities
  - US EPA-ORD

**67 locations twice a week**

**Sequencing to screen for possible presence of SARS-CoV-2 variants**
- Variants of Concern (VOC)
- Variants of Interest (VOI)

https://coronavirus.ohio.gov/wps/portal/gov/covid-19/dashboards/other-resources/wastewater
Public Health Application

- To serve as an early warning of infection in communities and an understanding of case trends
- The focus is on trends or significant changes in the number of viral gene copies detected.
- Currently action is taken when at least 3 samples show a sustained increase of at least 10-fold (1 log)
- State actions when increases are observed:
  - Notify the local health district and utility
  - Provide information on how to interpret the data and link to message toolkit
  - Notify the state pandemic testing team for linkages to establish pop-up testing sites
  - Provide case data by sewershed to local health district (this extraction to be provided soon)

- Participation in the CDC National Wastewater Surveillance System
Accomplishments

- Built statewide network that represents wastewater flow from nearly 5 million residents
- Almost 1 year of weekly data collected
- All data is publicly available on the Ohio coronavirus dashboard and is updated daily
- Provided nearly 500 warnings to local health communities
- Expanded to include genomic sequencing of wastewater to pair with clinical data and inform public health decisions
• Twice weekly samples
• Report data within 2 days of sample receipt
• No prescribed method; labs decide
• Supply chain shortages
• Low target concentration
• Sample hold time: 4°C - 72 hours
• No sample pasteurization
• **Matrix Spike to assess method recovery efficiency**
  – Coronavirus recommended: human (OC43), murine (MHV), bovine (BCoV)

• **Inhibition control to monitor for PCR amplification inhibition**

• **RT-qPCR standards/RT-ddPCR positive control**

• **Human fraction measurements**
  – crAssphage
  – Pepper mild mottle virus

• **Monthly Interlaboratory Method Validation**
  – Pick a site with sufficient concentration of SARS-CoV-2
  – Each lab gets 0.5 L
  – Each lab processes and analyzes sample
  – Report data to Project Coordinator
<table>
<thead>
<tr>
<th>LAB</th>
<th>LOD (copies/L)</th>
<th>Processing Method</th>
<th>Nucleic Acid Extraction</th>
<th>Quantitative Analysis Method</th>
<th>RT-PCR Standard Curve/ Control</th>
<th>Inhibition Control</th>
<th>Matrix Spike</th>
<th>Fecal Indicator</th>
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<tbody>
<tr>
<td>A</td>
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<td>Centrifugation, filtration</td>
<td>Qiagen RNeasy PowerWater Kit</td>
<td>RT-qPCR</td>
<td>DNA plasmid</td>
<td>Dilution</td>
<td>MHV</td>
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<td>Centrifugation, filtration</td>
<td>Qiagen Allprep DNA/RNA Kit</td>
<td>RT-qPCR</td>
<td>DNA plasmid</td>
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<td>Tween, solids removal, hollow fiber ultrafiltration (InnovaPrep)</td>
<td>Qiagen PowerMicrobiome Kit</td>
<td>RT-ddPCR</td>
<td>DNA plasmid</td>
<td>Luciferase Control RNA</td>
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<td>Filtration</td>
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<td>Centrifugation, filtration</td>
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<td>Promega, add protease, supernatant through GFA/silica column</td>
<td>Promega Wastewater Large Volume TNA Capture Kit</td>
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<td>DNA plasmid</td>
<td>Promega probe</td>
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<td>Centrifugation, filtration</td>
<td>Qiagen RNeasy PowerWater Kit/Trizol-chloroform</td>
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<td>SARS-CoV-2 genomic RNA</td>
<td>Luciferase Control RNA</td>
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<td>Acidification, Filtration, extract filter</td>
<td>Qiagen Allprep PowerViral DNA/RNA Kit</td>
<td>RT-qPCR</td>
<td>Synthetic RNA</td>
<td>Mouse lung RNA</td>
<td>OC43</td>
<td>PMMoV</td>
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## OH Network Lab Methods

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Number of different procedures employed at the various processing/analysis steps.
N2 RNA concentrations from 8 labs span >2 orders of magnitude
RNA sequences with deletions of nucleotides that result in absence of spike aa 69-70 increases over time

RNA sequences with nucleotides that change spike aa 501 increases over time
• **Pooled sample**
  - Cannot assemble a genome
  - Focus on mutations that cause amino acid substitutions, signatures of VOC/VOI

• **Genome/Genetic Sequencing**
  - 3 labs, different methods
  - Tiled amplicon approach
  - Short read seq via Illumina
  - Short term - spike amino acid changes for CDC’s VOC/VOI
  - Report (for each site)
    • Read depth
    • Number of alternative alleles
• How do current practices (eg. methods, protocols, technologies, best practices, etc.) successfully contribute toward comparable, high quality data/results/decisions?
  – Using a consistent method, trends of SARS-CoV-2 RNA in a sewershed can be evaluated
  – Implementation of Quality Control parameters allow for confidence in lab measurements
  – Frequent communication/regular meetings facilitate interlab discussion and troubleshooting

• How do current practices compromise efficiency and reduce confidence in data/results/decisions?
  – Too many labs/methods result in measurement variation
  – Varied experience leads to measurement variation
  – Supply shortages lead to method changes

• What is needed to increase comparability and confidence in data and results?
  – Standardized methods/procedures
  – Standardized quality control samples/reagents
  – Statistical models to quantify uncertainty

• What types of standards could potentially help to fill these needs?
  – Matrix Spike
  – Extraction controls
  – RT-qPCR standards
  – RT-ddPCR controls
  – Inhibition controls
  – Sequencing controls
Research Team and Partners

**EPA/ORD**
- Maitreyi Nagarkar
- Chloe Hart
- Scott Keely
- Emily Wheaton
- Michael Jahne
- Eunice Varughese
- Jay Garland
- Brian Morris
- Ana Braam
- Barry Wiechman
- Sara Okum

**Utilities**
- Metropolitan Sewer District of Greater Cincinnati
  - Bruce Smith
- City of Dayton
  - Chris Clark, Walter Schroder
- City of Marion
  - Brittany Bauer
- City of Portsmouth
  - Tommy Stewart
- Montgomery County
  - Jim Davis
- City of Hamilton
  - Mark Smith
- City of Springfield
  - Jeff Yinger

**Hamilton County Public Health Department**
- Chris Griffith

**Ohio Water Resources Center**
- Zuzana Bohrerova

**Ohio Department of Health**
- Rebecca Fugitt
- Brian Hall
- Tiffani Kavalec

**University Labs**
- Ohio State University
- University of Toledo
- Kent State University
- University of Akron
- Bowling Green State University

**Commercial Lab**
- LuminUltra